DATE DUE

HA   N EMISSIONS FROM VOLCANOES

NEXT WEEK IN NATURE

Solar neutrinos reviewed ■ Optical counterpart for eclipsing
pulsar ■ New 120K superconductor ■ Earthquake pre-
cursors ■ Cellular sygnals ■ Virulence of plague bacterium
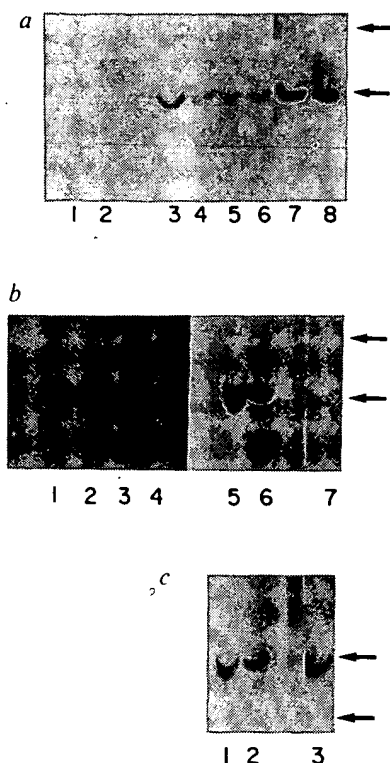■ Molecular evolution ■ *Pneumocystis carinii* a fungus?

Fig. 3 Detection of the yeast ATPase gene in cells transformed by plasmid pSV$_h$AT$_5$. High molecular weight DNA (20 μg) obtained from the different cell lines and tumours was digested with HindIII, subjected to electrophoresis, blotted and hybridized with a ^{32}P-labelled, nick-translated 4.2-kb ATPase sequence from plasmid pSV$_h$AT$_5$. a, Lanes 1 and 2 correspond to the control cell lines Vneol and Neol, respectively; lanes 3-8 correspond to the ATPase-expressing lines RVt3a, RVt3b1, RVle3, RNt3a, RN1a and RN1ds in the indicated order (see Table 1). b, Lanes 1-4 contain pSV$_h$AT$_5$ plasmid digested with HindIII in amounts equivalent to 50, 10, 5 and 1 gene copies per genome, respectively; the other lanes contain DNA from tumours induced in Swiss (lane 5) and nude (lane 6) mice by cell line RN1ds, its DNA being analysed in lane 7. c, Same blot as above, washed and rehybridized with a 1.5-kb EcoRI fragment of the Mac-1 mouse gene[12]. The upper and lower arrows correspond to size markers of 23 and 4.4 kb, respectively.

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Extensive variation of human immunodeficiency virus type-1 in vivo

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Genotypic variation among independent isolates of human immunodeficiency virus type-1 (HIV-1) is well known[1-9], but its molecular basis and biological consequences are poorly understood. We examined the genesis of molecular variation in HIV-1 by sequential virus isolations from two chronically infected individuals and analysis of recombinant HIV-1 genomic clones. In three different virus isolates full-length HIV-1 clones were identified and found to consist, respectively, of 17, 9 and 13 distinguishable, but highly-related, viral genotypes. Thirty-five viral clones derived from two HIV-1 isolates obtained from the same individual but 16 months apart showed progressive change, yet were clearly related. Similar changes in the HIV-1 genome did not occur in vitro during virus isolation and amplification. The results indicate that HIV-1 variation in vivo is rapid, that a remarkably large number of related but distinguishable genotypic variants evolve in parallel and coexist during chronic infection, and that 'isolates' of HIV-1, unless molecularly or biologically cloned, generally consist of complex mixtures of genotypically distinguishable viruses.

In previous studies, we observed that independent as well as sequential HIV-1 isolates from individual patients showed evidence of genetic change[1,2,5,8]. Genotypic analysis of paired virus isolates from donor-recipient transfusion cases[10], and nucleotide sequence comparisons of geographically distinct HIV-1 strains[4], provided further evidence for the highly variable nature of HIV-1. The extent and patterns of HIV-1 variation in chronically infected individuals, however, were not known. To address this question, we examined sequential virus isolates from two individuals (R.J.S. and W.M.F.)[8] at a molecular level. Recombinant lambda phage libraries were prepared from isolates RJS4, WMF1 and WMF3 so that individual complete viral DNA molecules, which together comprise the overall isolate DNA[8], could be analysed. A total of 27, 17 and 18 full-length HIV-1 clones were obtained from the three libraries and detailed mapping using 11 restriction endonucleases revealed that 17 of 27 RJS4 clones, 9 of 17 WMF1 clones, and 13 of 18 WMF3

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Fig. 1 Analysis of variation among HIV-1 DNA clones.

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                  HXB2 LAV-MAL LAV-ELI ARV2 WMJ1 | RJS4 a ... q        | WMF1 a ... i      | WMF3 a ... m

HXB2       —
LAV-MAL    63  —                                          55
LAV-ELI    62  58  —                               55              54                 56
ARV2       41  68  70  —
WMJ1       49  63  58  55  —

RJS4 a     46  61  67  49  49 | —
     b     45  63  66  47  47 | 11 —
     c     45  59  69  47  47 | 04 08 —
     d     44  62  68  46  46 | 11 07 11 —
     e     45  59  65  48  47 | 07 11 11 04 —              13
     f     47  66  65  50  50 | 15 04 12 11 14 —
     g     49  63  66  53  51 | 04 15 12 14 11 12 —
     h     46  64  67  49  49 | 15 11 11 04 07 08 11 —
     i     45  63  68  48  51 | 07 17 11 10 07 21 11 14 —
     j     42  61  70  45  49 | 07 11 04 11 14 15 11 14 07 —
     k     43  63  66  45  49 | 10 14 14 07 03 17 14 10 03 10 —
     l     45  66  65  48  49 | 14 17 17 10 07 14 11 07 07 14 03 —
     m     41  63  65  44  47 | 07 11 11 10 07 14 11 14 07 07 03 07 —
     n     45  63  62  51  51 | 12 08 15 14 11 12 15 18 17 18 14 17 11 —
     o     46  61  70  53  56 | 21 21 18 17 20 21 18 14 14 14 17 14 20 30 —
     p     51  59  66  41  47 | 08 22 15 21 17 25 15 24 11 11 14 17 11 21 24 —
     q     58  66  65  54  54 | 15 19 19 18 14 22 19 21 14 21 17 21 21 19 28 19 —

WMF1 a     46  57  59  53  49 | 36 39 39 38 35 42 39 41 40 41 37 40 35 39 46 39 42 | —
     b     45  59  58  51  47 | 34 38 38 36 33 41 38 39 38 39 35 38 33 38 44 38 41 | 04 —
     c     42  61  59  49  49 | 36 39 39 38 35 42 39 38 35 36 32 35 30 39 41 34 42 | 07 04 —         14
     d     46  60  63  49  53 | 42 44 44 43 41 47 44 46 41 42 38 41 36 44 46 40 47 | 10 14 10 —
     e     44  58  61  50  50 | 38 41 41 40 37 44 41 43 37 38 34 37 32 41 43 36 44 | 04 07 04 07 —       24
     f     51  62  64  54  54 | 43 46 46 44 42 49 46 46 46 44 43 46 42 46 51 46 49 | 11 14 17 07 14 —
     g     45  63  62  49  51 | 40 43 43 42 39 46 43 43 39 40 36 39 34 43 44 38 46 | 14 10 07 03 10 10 —
     h     49  63  62  53  51 | 40 43 43 42 39 46 43 44 43 44 41 43 39 43 49 43 46 | 14 10 13 10 17 07 07 —
     i     49  66  65  48  51 | 49 51 51 50 47 54 51 53 45 49 45 47 43 51 53 47 53 | 31 29 25 26 28 33 24 29 —

WMF3 a     54  57  59  56  53 | 41 44 44 43 40 47 44 44 46 42 44 40 44 50 44 47 | 14 18 21 23 17 23 26 26 31 | —
     b     55  58  61  57  54 | 43 46 46 44 42 49 46 47 46 47 43 46 42 46 51 46 49 | 17 21 24 25 20 27 28 28 33 | 04 —
     c     53  56  62  55  51 | 44 42 42 41 43 45 47 44 47 44 44 47 43 47 49 47 50 | 18 21 24 26 21 27 29 29 35 | 04 07 —
     d     51  58  61  54  54 | 38 41 41 40 37 44 41 43 42 43 39 42 37 41 47 41 44 | 11 14 17 19 14 20 23 23 33 | 04 07 07 —        13
     e     50  57  63  53  53 | 41 39 39 38 40 42 44 41 44 41 42 44 40 44 46 44 47 | 14 18 21 23 17 23 26 26 35 | 07 11 04 04 —
     f     53  56  58  55  51 | 39 42 42 41 38 45 42 44 43 44 40 43 38 42 49 42 45 | 11 15 17 20 14 21 23 23 29 | 04 07 08 07 11 —
     g     51  54  61  54  50 | 42 41 41 39 41 44 45 42 46 42 43 46 41 45 47 45 48 | 15 19 21 23 18 24 27 27 32 | 08 11 04 11 08 04 —
     h     51  58  64  54  54 | 43 41 41 40 42 44 46 43 46 43 43 46 42 46 47 46 49 | 17 21 23 19 20 20 23 23 38 | 11 14 07 07 04 14 11 —
     i     56  59  58  54  55 | 44 47 47 46 43 45 42 44 47 49 44 43 43 47 49 47 50 | 18 22 24 27 21 27 29 29 34 | 11 07 15 14 18 08 12 21 —
     j     51  58  62  59  54 | 43 46 46 44 42 49 46 47 42 43 39 42 37 46 47 41 49 | 17 21 17 19 14 26 23 28 28 | 11 14 14 14 17 07 11 20 14 —
     k     50  57  59  53  53 | 41 44 44 43 40 47 44 46 40 41 37 40 35 44 46 39 47 | 14 18 14 17 11 23 20 27 31 | 14 11 18 17 21 11 15 23 11 11 —
     l     55  58  65  58  54 | 47 45 45 44 46 48 50 47 50 47 47 50 46 50 51 50 53 | 21 25 28 29 24 30 32 32 38 | 08 11 04 11 07 12 08 11 19 18 21 —
     m     63  64  59  59  59 | 53 56 56 54 51 55 52 53 56 57 53 51 51 56 57 52 59 | 29 32 34 35 31 37 39 39 44 | 15 19 19 19 22 19 23 25 19 25 29 16 —
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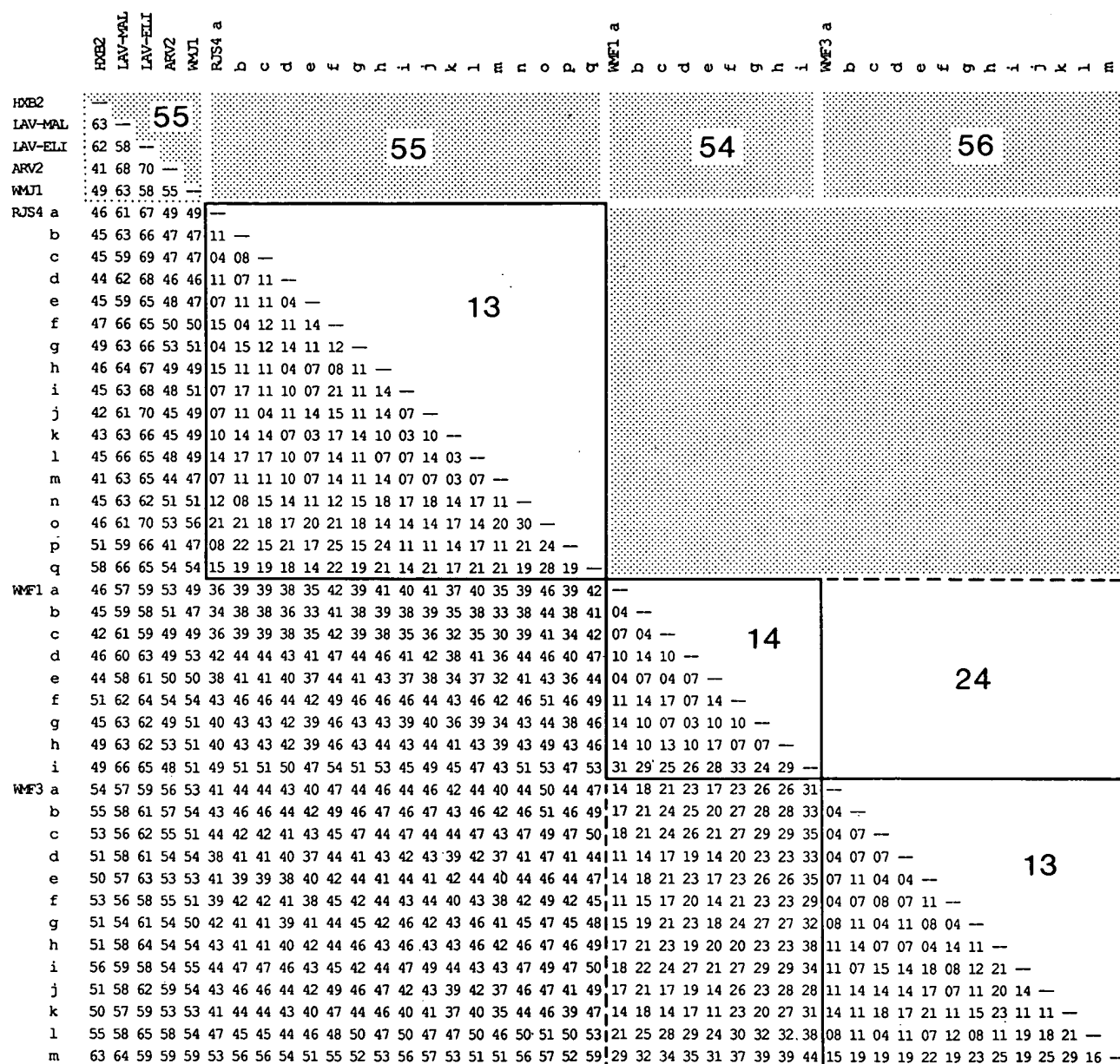
Fig. 1 Analysis of variation among HIV-1 DNA clones. Each of the distinguishable clone patterns depicted in Fig. 3 was compared pairwise to every other clone pattern, and the percentage difference in restriction endonuclease cleavage sites was calculated as follows: $(A+B)/C \times 100 =$ % restriction site differences, where, A equals the number of restriction sites present in one clone (X) that are missing in the other clone (Y); B equals the number of restriction sites present in clone Y that are missing in clone X; C equals the total number of restriction sites present in clones X and Y combined, with identical sites in the pair counted only once. For example, if clone X and clone Y have 25 restriction sites in common and no additional sites, they have: $(0+0)/25 \times 100 = 0\%$ restriction site differences. If clones X and Y each have 25 restriction sites but none are in common, they have: $(25+25)/50 \times 100 = 100\%$ restriction site differences. Because EcoRI and SstI enzymes were used to clone RJS and WMF, these enzyme sites were not included in the analysis of variation among these clones. Also, because the two terminal SstI sites in all clones except WMF1b, c, g, h, and i actually represent redundant sequences in their long terminal repeats, only one of the two sites was included in the analysis of variation. Boxed in solid lines are the difference scores for viral clones derived from the RJS4, WMF1 and WMF3 isolates. Dashed lines highlight the comparison of viral clones between isolates WMF1 and WMF3, and dotted lines highlight independent (unrelated) clones. Mean differences among clones from within individual isolates (RJS4a–q; WMF1a–i; WMF3a–m) compared with independent (unrelated) isolates were calculated from raw data values, not percentages, and are shown in the open and shaded areas.

clones were distinguishable by unique cleavage patterns (Fig. 3). These differences resulted from different combinations of restriction site polymorphisms, and, as expected, the restriction patterns of the most prevalent HIV-1 clones corresponded with the fragments visualized on Southern hybridizations of the primary isolate DNA[8]. Because each restriction enzyme generally gave only one to three different fragment patterns, this variation in viral genotypes within a single virus isolate has previously gone unrecognized.

The genomic restriction patterns (Fig. 3) showed that clones from individual virus isolates (RJS4a–q; WMF1a–i; WMF3a–m), despite their heterogeneity, were considerably more similar to other clones within the same isolate than to unrelated clones. To quantify this variation, we carried out a pairwise comparison of the percentage of restriction site differences between each of the 44 viral genotypes shown in Fig. 3 (total of 946 independent comparisons; Fig. 1). Such an analysis is a valid means for estimating overall nucleotide sequence variability[11,12]: assuming
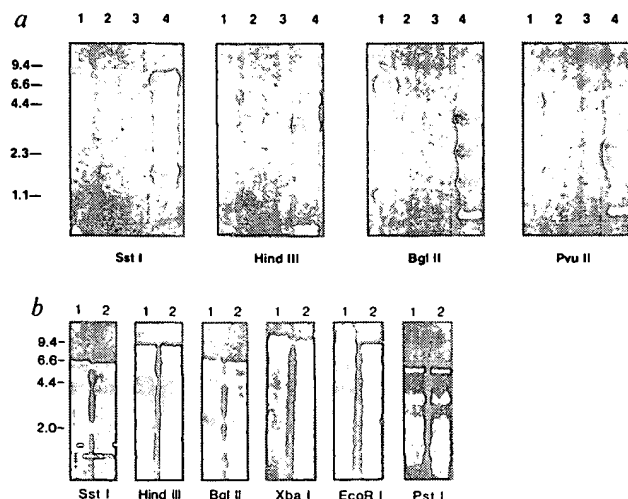
Fig. 2 Southern blot hybridization patterns of HIV-1 DNA comparing (a) uncultured versus cultured brain tissue specimens and (b) co-cultures of lymphocytes from an HIV-1 infected patient with normal lymphocytes from two HLA-unrelated donors (lanes 1 and 2). In a, lanes 2 and 4 contain DNA from uncultured and cultured brain tissue of an AIDS patient, lane 1 DNA from an unrelated HIV-1 isolate, and lane 3 normal human lymphocyte DNA.

Methods a, Brain tissue was obtained from an AIDS patient at autopsy and divided. Half was minced and co-cultured with interleukin-2 stimulated normal donor lymphocytes for four weeks to isolate and amplify HIV-1, and the other half was extracted immediately for high molecular weight DNA without interim culture. b, Identical aliquots of PBL from a different HIV-1 infected subject were co-cultured for four weeks with PBL from two HLA-unrelated donors. DNA extractions and blot hybridizations with full-length HIV-1 probe (pBH10i) were performed as described[1].

that the loss or gain of a restriction site in otherwise highly-related genomes results from a change in a single nucleotide, a 50% difference in restriction sites for enzymes that recognize six base pair sequences corresponds to about an 8% nucleotide sequence divergence (that is, 1 nucleotide change out of 12 nucleotides sampled). We used this approach first to analyse clones of HIV-1 for which nucleotide sequence information was available in the GenBank database, Los Alamos National Laboratory, and the correlation between predicted and observed nucleotide sequence differences was found to be quite good. For example, clone HXB2 differs from clones BH10, ARV2 and LAVeli respectively by 13%, 41% and 62% in restriction sites (ref. 1 and Fig. 1). Thus the predicted nucleotide differences are 2.2%, 6.8% and 10.3% and the actual differences determined by nucleotide sequence comparisons are 1.6%, 5.8% and 9.7%. Similarly, clones LAVmal, LAVeli and ARV2 (Fig. 1) differ by 58–70% in restriction sites, by 10–12% in predicted nucleotide sequence and by 10.1–13.0% in sequence determined experimentally (and deposited in GenBank).

The restriction site differences among viral DNA genomes comprising the individual isolates RJS4, WMF1 and WMF3 were considerably less than those of viral genomes representing unrelated (independent) isolates. For example, the 17 different RJS4 genotypes varied from each other by 3–28% (mean of 13%) whereas the same clones varied from independently isolated viruses by 41–70% (mean of 55%; $P < 0.0001$, chi-square analysis). The nine different WMF1 genotypes varied from each other by 3–33% (mean of 14%) and from independent viruses by 42–66% (mean of 54%; $P < 0.0001$). The 13 different WMF3 genotypes varied from each other by 4–29% (mean of 13%) and from independent viruses by 50–65% (mean of 56%; $P < 0.0001$).

Isolates WMF1 and WMF3 were derived from cultures of peripheral blood lymphocytes of the same individual taken 16 months apart. If the viral forms present in WMF3 had evolved from viruses present in WMF1, or from common precursor viruses in that patient, the extent of similarity between WMF1 and WMF3 viral genomes should be intermediate between values for clones from within each isolate, and clones from unrelated isolates. Conversely, if superinfection with unrelated viruses had occurred, genotypes of WMF1 and WMF3 viruses would be expected to vary to the same extent as the unrelated isolates HXB2, LAVmal, LAVeli, ARV2 and WMJ1. The data in Fig. 1 show that the viral clones of WMF1 (a–i) differed from clones of WMF3 (a–m) by 11–44% (mean of 24%), a range intermediate between values for viruses from within single isolates (mean of 13%; $P < 0.0001$) and from various unrelated isolates (mean of 55%; $P < 0.0001$). These data indicate that the viruses comprising WMF3 evolved either from genomes present in WMF1, or from precursor genomes common to both. The absence of clones in WMF3 that are identical to clones in WMF1, the existence of viral genotypes in WMF1 and WMF3 that differ in restriction pattern by 11–44% (2–7% estimated nucleotide sequence difference), and the fact that most viral species comprising an 'isolate' actually differ from the most prevalent genotypic form within the isolate (for example, RJS4a versus RJS4b–q), underscore the extremely rapid rate of change of HIV-1 *in vivo*.

As a control for these studies, a recombinant lambda library identical to those prepared for RJS4, WMF1 and WMF3 was made from an isolate of HIV-1 that had been biologically-cloned by five sequential cell-free end-point dilutions of culture supernatant. This was done to obtain a genotypically-pure virus stock that could then be expanded in culture (as is done during the isolation of HIV-1 from human tissues) to determine how much genotypic variation is introduced during virus amplification *in vitro*. From this library, ten full-length viral DNA clones were identified and found to be identical in 270 out of 273 restriction sites mapped (average of 27 sites per clone; data not shown). This minimal degree of restriction site variability in virus forced through multiple rounds of *in vitro* replication was much less than that of the isolates RJS4, WMF1 and WMF3, and could

▷

Fig. 3 Restriction endonuclease cleavage patterns for seven independent HIV-1 viral clones (HXB2; LAVmal; LAVeli; ARV2; WMJ1; RJS4a; WMF1a) and for distinguishable viral clones from isolates RJS4, WMF1 and WMF3 (ref. 8). Shown to the right of each set of maps is a summary of the different restriction patterns (E, *Eco*RI; S, *Sst*I; X, *Xho*I; B, *Bgl*II; U, *Pvu*II; H, *Hind*III; P, *Pst*I; K, *Kpn*I; M, *Bam*HI; C, *Cvn*I; A, *Xba*I) with the top clone pattern designated 1111111111 and differences in subsequent clone patterns identified sequentially. The laboratory clone designation (for example, RJS4.1; RJS4.11; RJS4.16) and the total number of viral clones represented by each pattern are indicated.

Methods. Isolates RJS4, WMF1 and WMF3 were derived from peripheral blood lymphocytes (PBL) of HIV-1 infected individuals as previously described[8]. Non-amplified recombinant phage libraries were prepared in λ-gt Wes/λ B and screened with [³²P]BH10i (a full-length HIV-1 probe) by standard techniques[1]. RJS4 was cloned in permuted form by digestion of circular DNA intermediate forms using *Eco*RI. WMF1 and WMF3 were cloned from integrated and unintegrated viral DNA in non-permuted form using *Sst*I. All 62 full-length HIV-1 clones that were identified were mapped side by side using Southern blot hybridization, single, double and triple enzyme digests, and full-length, 5' and 3' HIV-1 probes as described[1]. Clones comprising WMF1b, c, g, h and i patterns are ~170 base pairs shorter than the other clones due to a polymorphic *Sst*I site located in the 5' leader sequence[1]. The restriction maps of HXB2, LAVmal, LAVeli, ARV2 and WMJ1 were obtained from their nucleotide sequences (GenBank) or from previous publications[8].

Virus	Pattern										
	E	S	X	B	U	H	P	K	M	C	A
HXB2	1	1	1	1	1	1	1	1	1	1	1
LAVmal	2	2	2	2	2	2	2	1	2	2	2
LAVeli	3	3	1	3	3	3	3	2	3	3	3
ARV2	4	1	1	4	4	4	4	2	4	4	4
WMJ1	5	3	1	5	5	5	5	1	4	5	1
RJS4a	4	1	2	6	1	6	6	1	1	6	5
WMF1a	4	2	1	6	6	7	7	1	5	7	5

Virus	Pattern											Clone	Total
RJS4	E	S	X	B	U	H	P	K	M	C	A		Number
a	1	1	1	1	1	1	1	1	1	1	1	1, 11, 16, 39, 46	5
b	1	1	1	1	2	2	2	1	1	1	1	2, 23, 26	3
c	1	1	1	1	1	1	2	1	1	1	1	18, 34	2
d	1	1	1	1	1	2	2	2	1	1	1	15, 40	2
e	1	1	1	1	1	2	1	2	1	1	1	20, 24	2
f	1	1	1	1	2	2	2	1	1	2	1	12, 31	2
g	1	1	1	1	1	1	1	1	1	2	1	7	1
h	1	1	1	1	2	2	2	1	2	1	1	32	1
i	1	1	1	2	1	1	1	2	1	1	1	42	1
j	1	1	1	2	1	1	2	1	1	1	1	6	1
k	1	1	1	2	1	2	1	2	1	1	1	22	1
l	1	1	1	2	1	2	1	2	1	2	1	14	1
m	1	1	1	2	1	2	1	1	1	1	1	21	1
n	1	1	1	1	2	2	3	1	1	1	1	45	1
o	1	1	1	2	1	3	2	2	1	2	2	19	1
p	1	1	1	2	3	1	1	1	2	1	1	43	1
q	1	1	1	3	2	1	1	2	2	1	1	4	1

Virus	Pattern											Clone	Total
WMF1	E	S	X	B	U	H	P	K	M	C	A		Number
a	1	1	1	1	1	1	1	1	1	1	1	4, 5, 9, 15, 16, 18	6
b	1	2	1	1	1	1	1	1	1	1	1	14, 19	2
c	1	2	1	2	1	1	1	1	1	1	1	11, 13	2
d	1	1	2	2	1	1	1	1	1	1	2	1, 12	2
e	1	1	1	2	1	1	1	1	1	1	1	10	1
f	1	1	2	1	1	1	2	1	1	1	2	2	1
g	1	2	2	2	1	1	1	1	1	1	2	6	1
h	1	2	2	3	1	1	1	1	1	1	2	3	1
i	1	2	1	4	2	1	1	1	1	2	3	17	1

Virus	Pattern											Clone	Total
WMF3	E	S	X	B	U	H	P	K	M	C	A		Number
a	1	1	1	1	1	1	1	1	1	1	1	1, 110, 111	3
b	1	1	1	1	1	1	1	2	1	1	1	103, 107	2
c	1	1	1	1	1	1	2	1	1	1	1	3, 108	2
d	1	1	1	1	2	1	1	1	1	1	1	10, 104	2
e	1	1	1	1	2	1	2	1	1	1	1	102	1
f	1	1	1	2	1	1	1	1	1	1	1	11	1
g	1	1	1	2	1	1	2	1	1	1	1	101	1
h	1	1	2	1	2	1	1	1	1	1	1	106	1
i	1	1	1	2	1	1	1	2	1	2	1	9	1
j	1	1	1	3	1	1	1	1	1	1	1	7	1
k	1	1	1	4	1	1	1	2	1	1	1	12	1
l	1	1	1	1	1	1	2	3	1	1	1	105	1
m	1	1	1	3	1	1	4	1	2	1	1	109	1
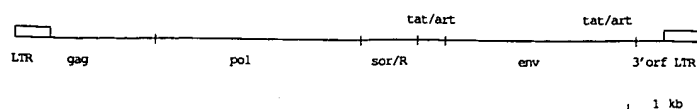
even have resulted from incomplete biological cloning of the virus stock before final amplification. In a second set of experiments (Fig. 2), viral DNA obtained from cultured versus uncultured tissue specimens, and from parallel co-cultures of patient P.B.L. with lymphocytes from different normal donors, were found to have identical genotypic patterns. These data suggest that virus populations isolated by short-term culture of patient tissues are generally representative of viral populations present *in vivo*, although it is well established that under conditions of selective pressure genetic changes in the HIV-1 genome can occur *in vitro*[13].

Previously, we mapped and sequenced predominant viral clones representing three sequential HIV-1 isolates (WMJ1, WMJ2, WMJ3) from a child with AIDS and calculated the rate of viral evolution to be on the order of 10^{-3} nucleotide substitutions per site per year[8]. The current study extends these findings by demonstrating rapid, parallel evolution of large numbers of related but distinguishable HIV-1 genotypes during chronic viral infection. These data are similar to those reported for equine infectious anaemia virus (EIAV) in which parallel evolution of multiple genotypic and antigenic variants has been documented in experimentally infected animals[14,15]. The potential molecular mechanisms underlying HIV-1 variation have been discussed[4-9]. It is of interest and potential clinical relevance that in none of the RJS, WMF or WMJ genomic libraries, nor in a large number of virus isolates from multiply exposed high-risk individuals[1,2], was there evidence for concomitant superinfection with HIV-1 strains that were genotypically unrelated to the predominant viral forms. Whether this was due to selective pressures of *in vitro* cultivation or to *in vivo* mechanisms that protect against superinfection is unknown.

The biological and immunological significance of HIV-1 variation in viral pathogenesis is currently uncertain, but there are indications that similar variation in FeLV[16], EIAV[14,15,17] and visna[18,19] is important. There are also reports that some isolates of HIV-1 have a preferential tropism for mononuclear phagocytes[20,21], and, more recently, that more virulent forms of HIV-1 may become apparent as clinical immunodeficiency progresses[22]. Ten of 12 clones of RJS4, WMF1 and WMF3 that were tested for transfection competence gave virions that were morphologically indistinguishable from wild type, but which expressed biological phenotypes ranging from highly cytopathic lymphotropic viruses, to forms that replicated selectively in monocytes or not at all (A. Fisher, L. Kong and G. M. S., unpublished data). Future studies examining the genetic, biological and immunological properties of HIV-1 will need to account for the extensive variability of this virus.

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Biologically diverse molecular variants within a single HIV-1 isolate

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AIDS is a disorder characterized by a slow progressive impairment of immune function and by infection of human immunodeficiency viruses (HIV-1, HIV-2)[1-4]. Our knowledge of how these viruses cause disease in man, or how the related lentiviruses (visna and equine infectious anaemia virus) cause disease in animals, is still fragmentary. In particular, the significance of genetic variation in HIV-1, occurring within populations, within individuals and over periods of time[5,6], and the mechanisms of viral persistence remain unclear. To address these issues we prepared a series of proviral clones of HIV-1 originating from a single patient and compared their biological properties. Here we show that hybrid genomes (in which the envelope region of six viral clones were separately substituted into a prototype HIV-1 genome) generated viruses with widely differing capacity to grow in human T cells, cell lines and monocytoid cultures. These data suggest that extensive biological variation exists *in vivo* within an infected individual and is in part determined at the level of the viral envelope.

Virus was isolated from the peripheral blood of an HIV-1 infected individual (coded R.J.S., HIV-1 isolate 4), who was a promiscuous homosexual male, chronically infected with HIV-1 for 5 years. Previous analyses had shown that serial isolates from R.J.S. were highly related and comprised a mixture of similar but distinct viral substrains[7]. Molecular clones were made of 27 full length proviral forms from isolate 4 and these were found to represent at least 17 different prototypes[8]. Of these, six clones were selected for further study (numbers 6, 15, 16, 22, 24 and 26) as detailed in Fig. 1. Because these proviruses were cloned using *Eco*RI (an enzyme which severs the viral genome)[8] and thus cloned in a permuted form, we isolated *env*-containing fragments (nucleotides 5,364-8,054) from each clone and placed these into the corresponding sites in the biologically active molecular clone pHXB2gpt[9]. This, we reasoned, would provide a panel of related viruses (designated JS4) in which the effects of envelope variation could be measured independently of the effects created by differences in other areas of the genome. It was also hoped that the constituents of such a panel would serve as useful reagents for neutralization studies. As shown in Fig. 1, each of the hybrid JS4 clones generated morphologically normal virus particles (Fig. 1c-1j) and high levels of reverse transcriptase upon transfection into the COS-1

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